COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Toxicological evaluation of novel heat-not-burn tobacco products: preliminary review of additional literature from sources not associated with product manufacturers and developers.

Introduction

1. This addendum provides further detail on the papers which were not reviewed in time for the main TOX/2017/42 paper, and should be considered in conjunction with the data provided there.

2. For the Committees review of heat-not-burn tobacco products, a review of the literature on HNB tobacco products published by sources that are independent of the developers/manufacturers of these products was requested. A literature search was performed and the publications identified are reported in the main paper TOX/2017/42.

3. An additional literature search was carried out from which an additional four publications were identified and reported in this addendum paper.

Question for the Committee

i. Is there any information in the independent literature summarised in this addendum paper which should be incorporated in the second draft statement?

Abbreviations

BC	Black carbon
CC	Conventional cigarette
CO	Carbon monoxide
CSE	Cigarette smoke extract
DC	Dendritic cells
EC	e-cigarettes
Ec	Eclipse cigarettes
EF	Emission factors
HNB	Heat-not-burn
HO-1	Heme-oxygenase-1
IL	Interleukin
IOM	Institute of Medicine
IQOS	I quit ordinary smoking
LPS	Lipopolysaccharide
MRTP	Modified risk tobacco products
PAH	Polycyclic aromatic hydrocarbon
PBS	Phosphate buffered saline
PM	Particulate matter
QSU	Questionnaire of smoking urges

Details of the literature searches

4. As described in the main paper (TOX/2017/42), a literature search was performed by Imperial College London under contract to PHE on 01/06/2017, with the aim of identifying reports relating to HNB products that were not conducted or sponsored by developers or manufacturers of these products. In total five papers were identified as being independent publications, which have been discussed in the main paper (TOX/2017/42).

5. A further literature search was performed by National Centre for Environmental Toxicology at WRc (NCET at WRc) and IEH-Consulting (IEH-C) (NCET at WRc/IEH-C) under contract to PHE on 01/08/2017 using additional search terms and additional literature databases. The details of this search are described in Annex A. Four publications were identified as being independent publications on HNB tobacco products, namely Ruprecht et al. (2017), Vassallo et al. (2015), Institute of Medicine (2012) and Buchhalter and Eissenberg (2000).

Independent publications on HNB tobacco products (to 01/08/2017)

6. In a poorly reported study, Ruprecht et al. (2017) evaluated the indoor environmental pollution generated by heat-not-burn (HNB) cigarettes (IQOS, menthol and non-menthol types) in comparison with conventional cigarettes (CC) (type/brand not stated) and e-cigarettes (EC) (Elips Serie C, Tank System, Ovale Europe Srl). Testing comprised the use/smoking of the specific product for around 3 h (consisting of repeated cycles of approximately 7 min use + 3 min pause) in a 'standard indoor environment' (48 m^2 apartment of regular smokers) with fans to circulate the air and sampling 2 m from the smokers/product users. For EC, results were reported as the average of 13 'vaping' (1 puff/min for 7 min followed by a 3 min pause, during 2-3 h) sessions. For IQOS, a total of 10 non-menthol and 14 menthol sticks were tested, with each use session lasting around 3 h (7 min use followed by 3 min pause). A CC smoking session comprised smoking 9 cigarettes (for each, approximately 7 min smoking followed by 3 min pause). For EC and CC, measurements of particulate metals and organics were taken from a previous study (Saffari et al., 2014), in which measurements were made inside (-/+ product use) and on the outside terrace (concurrent ambient air measurements) of a fifth-floor office room in Milan, Italy.

7. Real-time air sampling was carried out for black carbon (BC) and particulate matter (PM), and compared with background indoor air levels in the same apartment (measured immediately before and 3 h after each test session). Smoking CC significantly increased levels of BC (measured at 880 nm ['soot'] and 370 nm [light-absorbing organic compounds]), PM (dp > 1.0 µm, dp > 0.3 µm) and sub-micronic particles (PMnm, 10-1000 nm) (both number and mass) compared with background. With IQOS use, a measurable increase over background in BC 370 nm (but not BC 880 nm) was detected (approximately 0.8% of the level measured during CC smoking) while no BC increase was detected during EC use. Small, non-significant increases in PM > 1.0 and PM > 0.3 were associated with EC use. IQOS use produced temporary, sharp increases in PM > 1.0 and PM > 0.3 (significant at p <

0.05 for PM > 0.3 only). PMnm were increased with both EC and IQOS use (to around 6% and 23%, respectively, compared with CC levels).

8. Levels of aldehydes (acrolein, acetaldehyde, formaldehyde), metals, and particle-phase organic compounds were determined by time-integrated chemical analyses and compared with levels measured concurrently in ambient outdoor air. All three aldehydes were present at higher levels in indoor air during CC smoking and IQOS use compared with outdoor ambient air levels. The indoor air levels of acrolein, acetaldehyde, and formaldehyde during IQOS use were approximately 2%, 5% and 7%, respectively, of those during CC smoking. Acetaldehyde and formaldehyde (but not acrolein) were higher in indoor air during EC use than in outdoor ambient air. The indoor air levels were approximately 0.3% and 3%, respectively, compared with indoor air levels during CC smoking. Some metals (Al, S, K, Ti, Sn) were produced at higher levels from menthol than non-menthol IQOS use, but there were no statistically significant differences between indoor and outdoor air levels of metals (grouped) for IQOS menthol or for IQOS non-menthol. PAHs, hopanes and steranes were not detected with IQOS use. n-alkanes, organic acids and levoglucosan in indoor air during IQOS use were higher (approximately 2x for n-alkanes and organic acids and between 8 to 20x for levoglucosan) than in outdoor ambient air. Levels of these substances associated with indoor EC use were lower than or comparable to those in outdoor ambient air.

9. Emission factors (EF) (number of particles or mass per unit time) were calculated for the various substances measured. Overall, these indicated that IQOS emitted small (< 1% of those emitted by CC) amounts of BC 370 nm (presumed by the authors to be n-alkanes and organic acids). Both IQOS and EC had measurable PMnm emissions (around 40% and 7%, respectively, of those emitted by CC). IQOS EFs for aldehydes were around 1.5% (acrolein), 3.5% (acetaldehyde) and 4.5% (formaldehyde) of those for CC. For EC, the EF for formaldehyde was approximately 2% of that from CC, while the EFs for the other aldehydes were low or non-detectable. Overall, emissions of organic compounds from IQOS and from EC were at lower levels than from CC. The most abundant organic compounds in IQOS emissions were hexadecanoic acid and linoleic acid (which can be calculated as approximately 7% and 14%, respectively, compared with CC).

10. The authors noted that, apart from aldehydes, the study only investigated particle-phase organics; given that a range of emissions from IQOS and EC may be in the gas phase, they suggested that this should be addressed in future studies. They also noted that the comparisons in this study were limited by the use of a single representative brand of each HNB and EC product. However, based on their findings they concluded that ‘smoking/vaping in indoor public environments should be restricted’.

11. Vassallo et al. (2015) investigated the relative toxicity of mainstream extracts from an HNB cigarette (EclipseRM, RJ Reynolds Tobacco) and a reference CC (3R4F University of Kentucky reference cigarette) on antigen-presenting cell function

in vitro. Extracts were generated using an apparatus by which 35 ml of the 3R4F (R) smoke or Eclipse (Ec) usage product were collected in a plastic tube and bubbled into 30 ml PBS at 37 °C. One 'cigarette' was smoked per 10 ml PBS. The extract solution was passed through a 2 µm filter and used immediately after preparation. It is unclear from the methodology described whether the same collection protocol was applied to the Ec product. For the discussion below it is assumed the term 'cigarette' applies to both products.

12. Nicotine concentrations measured in a 1% solution of the PBS product extract (termed 'cigarette smoke extract' [CSE] by the authors) were 174 ng/ml (R) and 453 ng/ml (Ec).

13. Four sets of *in vitro* tests were performed to evaluate the relative effects of the two CSEs on dendritic cell (DC) and macrophage viability and function, namely cell toxicity, suppression of interleukin-12 (IL-12) production, induction of oxidative stress and effects on global gene expression.

14. Cell toxicity: Human monocyte-derived DCs incubated with CSEs in culture medium for 24 h were assayed for percentage viable/necrotic/apoptotic cells using a commercially available Annexin V-PI kit. Viability was arbitrarily defined as ≥ 90% viable cells without Annexin V or PI positivity. For both R and Ec, > 90% of cells were viable at 2% CSE while < 90% of cells were viable at ≥ 4% CSE. To maintain cell viability, CSE concentrations ≤ 3% were used for subsequent experiments.

15. Suppression of IL-12 production: IL-12 levels in cell-culture supernatants of activated human monocyte-derived DCs or RAW 264.7 murine macrophage cells incubated with CSEs for 24 h were measured by ELISA. Ec-CSE (at 1%, 2%, and 3%) suppressed IL-12 production by human DCs to a greater extent than respective equivalent R-CSE concentrations (Anova, $p < 0.01$). There were no significant differences in magnitude of effect between Ec-CSE and R-CSE (at 1% or 2%) on suppression of IL-12 production by mouse macrophages (Anova, $p > 0.05$).

16. Induction of oxidative stress: Intracellular heme-oxygenase 1 (HO-1) levels, as a surrogate for cellular oxidative stress, were measured by immunoblotting from human monocyte-derived DC or murine macrophage lysates after incubation with 2% CSE for 6 h. Human DCs incubated with Ec-CSE had significantly higher HO-1 levels than those incubated with R-CSE (Anova, $p = 0.002$). Similar effects were seen in experiments using mouse macrophages (Anova, $p = 0.001$). The effects of HO-1 induction were abrogated by pre-incubation with anti-oxidant.

17. Effects on global gene expression: Gene expression profiles from LPS-stimulated murine bone marrow-derived DCs incubated with 2% CSEs for 4 h were evaluated using Affymetrix Mouse 430 2.0 arrays. No significant differences in gene expression profiles were observed for an a priori selected set of 271 immunity-related genes after exposure to R-CSE or Ec-CSE.

18. Vassallo and colleagues concluded that extracts from the Eclipse HNB cigarette did not show reduced toxicity, and moreover were associated with increased levels of oxidative stress, compared with the 3R4F reference CC. They noted that their study did not provide insight into the primary constituents in either Eclipse or 3R4F CSE responsible for the effects observed. Previous studies had indicated that nicotine concentrations of around 50 ng/ml or greater decrease IL-12 production from DCs, but also that the effects of oxidative stress on IL-12 suppression may be more important than those of nicotine. Vassallo et al. contended that an important factor in the outcome of experiments evaluating CSEs is the extract preparation procedure. With regard to this, while other authors had previously reported that Eclipse extracts induced lower levels of adverse effects (mutagenicity, cytotoxicity, DNA adducts, induction of genes associated with cellular stress and inflammation) than smoke condensate from reference CCs (Brown et al., 1998; Fields et al., 2005), these previously reported studies had followed extraction procedures that are likely to eliminate transient and volatile agents, which may in fact be relevant to tobacco-induced toxicity.

19. The US Institute of Medicine 'Committee on Scientific Standards for Studies on Modified Risk Tobacco Products' reported an in-depth evaluation of scientific standards for studies on modified risk tobacco products (MRTPs) (IOM, 2012). Approximately 20% of the adult population in the US are smokers: approximately 45% of these people attempt to quit smoking each year, and approximately 6% achieve this for ≥ 1 month. Some smokers turn to the use of products that are marketed as having less risk (MRTPs), and it is necessary that such claims of reduced risk are founded. A wide range of data are required, including: product composition, human exposure, human health effects (users and non-users), potential for addiction and abuse, public perception of the product including how marketing may affect users who may otherwise have quit, nonusers initiating product use and risks of use in comparison with other smoking-cessation products. With the premise that there is a need to develop guidelines for the evaluation of MRTPs, the Committee discussed evidence related to evaluating health effects, addictive potential, and risk perception and communication of MRTPs. They concluded that there is a shortage of credible and reliable evidence about the effects of MRTPs on individual and public health, and that trust in the ability of the industry to produce rigorously conducted studies has been undermined by previous behaviour of the industry. The report describes the Committee's evaluations and conclusions in detail, leading to a list of 12 recommendations for minimum standards/ requirement of evidence to support the marketing of MRTPs in the US.

20. Buchhalter et al. (2000) evaluated subjective and physiological aspects of using the 'Accord' (Philip Morris) HNB device in comparison with smoking CC. The study was carried out in two stages. During each stage, at 30-min intervals during a 2-h period, 10 regular CC smokers of '≥ 10/day light or ultralight cigarettes' (any brand) either smoked one of their normal brand of cigarette or used one Accord device. Stage order was equalised across the group and all subjects abstained from smoking for at least 8 h prior to each stage. Subject-rated measures (cravings,

anxiety etc., assessed on a visual analogue scale using the questionnaire of smoking urges [QSU]) and physiological measures (heart rate, skin temperature, blood pressure, expired carbon monoxide [CO]) were recorded before, during (physiological except CO), and after (subject-rated, CO) smoking/use. Puff topography was also measured (volume, duration, number per smoking/use session, inter-puff interval). Compared with baseline (measured immediately before the smoking/HNB use session), subject-rated measures such as craving a cigarette were significantly reduced after smoking each CC ($p < 0.05$), but significant craving reduction was only achieved after use of the fourth Accord product (i.e. towards the end of the 2-h session). The magnitude of change observed for physiological measures (such as increased heart rate) was greater during CC smoking than during Accord use ($p < 0.05$). Expired CO was higher after compared with before smoking a CC, and values increased sequentially during the 2-h session. Conversely, use of the Accord product was not associated with increased expiration of CO. Puff topography was different with Accord (longer puffs, shorter inter-puff interval, greater puff volume) compared with CC. The study authors suggested that inadequate withdrawal suppression by Accord may lead to increased use of the system, which might decrease any benefit that the product may have as a substitute for CC smoking.

Summary

21. A literature search was carried out on 01/08/2017 to identify literature (in addition to papers identified from an earlier search on 01/06/17) on heat-not-burn (HNB) tobacco products published by sources independent of the developers and manufacturers of these products. Four publications were identified, including three investigative reports (evaluation of sidestream air; *in vitro* immunotoxicity studies; evaluation of acute subjective and physiological measures in human subjects using a heat-not-burn product) and a 370 pp expert committee report from the US Institute of Medicine summarising recommendations for minimum standards for studies gathering evidence to support the marketing of modified risk tobacco products.

22. The findings and authors' conclusions of identified literature are summarised in Table 1, overleaf.

Question for the Committee

i. Is there any information in the independent literature summarised in this addendum paper which should be incorporated in the second draft statement?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
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Table 1. Literature on heat-not-burn tobacco published by independent sources.

Ref.	**Report type**	**Products discussed / compared**	**Smoking /product use regime; methodology**	**Results**	**Author conclusions / commentary**
Ruprecht *et al*. (2017)	Study report – sampling of sidestream air	CC (details not stated); e-cigarette (Elips Serie C, Tank System, Ovale Europe Srl); HNB (IQOS, menthol and non-menthol)	3 hours in an indoor environment (cycles of approx. 7 min active smoking/product use followed by 3 min pause); Real-time air sampling for black carbon (BC) and particulate matter (PMs); Time-integrated chemical analyses for metals, aldehydes and particle-phase organic compounds	IQOS use was associated with increased levels of: BC measured at 370 nm (light-absorbing organic compounds) [approx. 0.8% cf CC]; PM > 0.3 µm [approx 5% cf CC], PM 10-1000 nm [approx 23% cf CC]; Aldehydes (acrolein [approx 2% cf CC], acetaldehyde [approx 5% cf CC], formaldehyde [approx 7% cf CC]); Particle-phase organic compounds (n-alkanes [approx 2x outdoor air], organic acids [approx 2x outdoor ambient air] and levoglucosan [8-20x outdoor ambient air]; but <u>not</u> PAHs, hopanes and steranes)	1. IQOS has substantially lower emissions of most toxic compounds compared with CC, but is not risk free. 2. There were detectable and significant emissions of organic compounds, including n-alkanes, organic acids and aldehydes. 3. The above findings warrant caution in the unregulated use of these products in spaces where passive exposure to can occur. 4. Future studies should also address gas-phase organic emissions.
Vassallo *et al*. (2015)	Study report – *in vitro* immunotoxicity of mainstream smoke/ product extract	CC (3R4F University of Kentucky reference cigarette) (R); HNB (Eclipse^{RM},	'Cigarette smoke extracts' (CSE) were generated using an apparatus by which 35 ml of	<u>Cellular toxicity (apoptosis and necrosis of human monocyte-derived DCs)</u> > 90% cell viability with 2% Ec-CSE or R-CSE; < 90% cell viability with ≥ 4% Ec-	1. The extract from the Eclipse HNB product did not show lower toxicity to antigen-presenting cells *in vitro* than the extract of a standard reference CC. 2. The extract from the Eclipse

		RJ Reynolds Tobacco) (Ec)	the 3R4F (R) smoke or Eclipse (Ec) usage product were collected in a plastic tube and bubbled into 30 ml PBS at 37 ^{o}C. One 'cigarette' was smoked per 10 ml PBS. The extract solution was passed through a 2 µm filter and used immediately after preparation.	CSE or R-CSE <u>Suppression of IL-12 production by APCs</u> In human monocyte-derived DCs, effects of Ec-CSE > R-CSE at equivalent % extract (1,2,3%); In mouse macrophages, effects equivalent for both CSEs (at 1 and 2%) <u>Induction of oxidative stress</u> Effects of 2% Ec-CSE > 2% R-CSE in both human monocyte-derived DCs (p = 0.002) and mouse macrophages (p = 0.001) <u>Effect on global gene expression (immunity)</u> Equivalent gene expression profiles of mouse bone-marrow-derived DCs incubated with 2% Ec-CSE or 2% R-CSE	HNB product induced more oxidative stress to human DCs and mouse macrophages *in vitro* than extract from a standard reference CC. 3. Previous studies which reported lower levels of toxicity of Eclipse versus CC may have used a collection technique that eliminated important volatile agents from the extract.
IOM (2012)	Report of Scientific Committee (371 pp.)	Modified risk tobacco products (MRTPs)	-	Summary of US Institute of Medicine scientific committee discussions to develop minimum standards for scientific studies on modified risk tobacco products	Recommendations for minimum standards for studies gathering evidence to support the marketing of MRTPs
Buchhalter *et al*. (2000)	Study report – evaluation of acute effects (physiological and psychological) of HNB product	CC (light or ultralight, any brand); HNB (Accord®, Philip Morris)	2 test sessions, in counterbalanced order for 10 regular smokers after 8-hour abstention from	<u>Subject-rated measures</u> Craving to smoke significantly reduced after smoking each CC. Use of Accord product only associated with significantly reduced craving after 4th product use.	The authors suggested that inadequate capability of the Accord product to suppress the desire to smoke may lead to increased product use, hence reduced benefit of using the product as a substitute for

	use vs. smoking CC in human smokers		smoking. Each session comprised either smoking 1 CC (smoker's usual brand) or using 1 Accord product, each 30 minutes during 2 hours (i.e. 4x)	<u>Physiological measures</u> Significantly greater increase in heart rate during CC smoking than Accord use. CO expiration increased after smoking each CC (and sequentially during the 2-hour period); no CO expiration increase associated with Accord use.	smoking CC.

CC, conventional cigarette; HNB, heat-not-burn; BC, black carbon; PM, particulate matter; PAH, polycyclic aromatic hydrocarbon; Ec, Eclipse; CSE, cigarette smoke extract; PBS, phosphate-buffered saline; APC, antigen-presenting cell; DC, dendritic cell; IOM, Institute of Medicine; MRTP, modified risk tobacco product; CO, carbon monoxide

References

Buchhalter AR, Eissenberg T (2000). Preliminary evaluation of a novel smoking system: effects on subjective and physiological measures and on smoking behavior. Nicotine and Tobacco Res., 2(1), 39-43.

IOM (Institute of Medicine) (2012). Scientific Standards for Studies on Modified Risk Tobacco Products. Washington, DC: The National Academies Press.

Ruprecht AA, De Marco C, Saffari A, et al. (2017). Environmental pollution and emission factors of electronic cigarettes, heat-not-burn tobacco products, and conventional cigarettes. Aerosol Sci. and Tech., 51, 674-684.

Vassallo R, Wang L, Hirano Y, et al. (2015). Extracts from presumed "reduced harm" cigarettes induce equivalent or greater toxicity in antigen-presenting cells. Toxicol., 335, 46-54.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Toxicological evaluation of novel heat-not-burn tobacco products: preliminary review of literature from additional sources not associated with product manufacturers and developers.

Details of Literature search carried out by NCET at WRc/IEH-C

The subsequent literature search was performed by NCET at WRc/IEH-C under contract to PHE on 01/08/17 using the following search terms in PubMed, Scopus and Web of Science. Terms related to tobacco manufacturers were not included.

- "heat not burn" OR "modified risk tobacco" OR "heat* tobacco" OR "electrically heat* cigarette" OR "electrically heat* tobacco"

Total no. of papers retrieved (for screening) = 95

Exclusion criteria

Papers that had any connection to a tobacco industry were excluded from further consideration and those that were not relevant to the study question, i.e. papers referring to test methodologies, regulatory aspects, growing of tobacco plants. Papers for which only abstracts or conference proceedings were available were also excluded from further evaluation.

From the 95 papers retrieved, 27 were deemed appropriate for further consideration based on the titles. Twelve papers were excluded as they were not independent from the tobacco industry. Six papers were excluded as only abstract or conference proceedings were available and all were associated with Philip Morris International. The nine remaining papers were obtained and reviewed, five of which were excluded as they were covered e-cigarettes, smokeless tobacco, conventional cigarettes (CC) or were not independent. The remaining four papers were included in this review.

Total no. of papers for further evaluation = 4.

List of papers for further consideration

- Ruprecht AA, De Marco C, Saffari A, et al. (2017). Environmental Pollution and Emission Factors of Electronic Cigarettes, Heat-Not-Burn Tobacco Products, and Conventional Cigarettes. Aerosol Science and Technology, 51(6), 674-684.

- Vassallo R, Wang L, Hirano Y, et al. (2015). Extracts from Presumed "Reduced Harm" Cigarettes Induce Equivalent Or Greater Toxicity in Antigen-Presenting Cells. Toxicology, 335, 46-54.

- Committee on Scientific Standards for Studies on Modified Risk Tobacco Products, Board on Population Health and Public Health Practice & Institute, o. M. (2012). Scientific Standards for Studies on Modified Risk Tobacco Products.

- Buchhalter AR & Eissenberg T (2000). Preliminary Evaluation of a Novel Smoking System: Effects on Subjective and Physiological Measures and on Smoking Behavior. Nicotine and Tobacco Research, 2(1), 39-43.

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